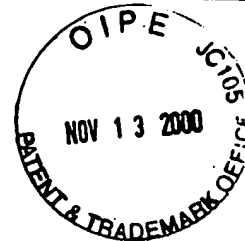


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## MODELLING HUMAN LEUKEMIA AND LYMPHOMA IN SEVERE COMBINED IMMUNODEFICIENT (SCID) MICE: PRACTICAL APPLICATIONS

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### INTRODUCTION

Mice homozygous for the severe combined immunodeficiency (SCID) mutation were first described by Bosma *et al.*,<sup>1</sup> following routine screening of a specific pathogen-free colony of C.B.-17/Icr mice. These mice are deficient in functional B- and T-lymphocytes and as a consequence can generate neither humoral nor cell-mediated immune responses. They do however retain normal myeloid cell differentiation and Natural Killer (NK) cell activity.<sup>2</sup> The defect is inherited as an autosomal recessive gene and is the direct result of inappropriate recombination of the B- or T-cell antigen receptors consequent to defective recombinase activity.<sup>3</sup> The severity of the immune defect in SCID mice allows for the xenotransplantation of both normal and malignant human hematological cells. Prior to the discovery of SCID mice, athymic nude mice served as an alternative for xenotransplantation of human hematological tumours but these models had limitations with tumours growing mainly as solid subcutaneous masses, very rarely producing a disseminated pattern of disease. It was soon established however, that human tumours transplanted into SCID mice generally disseminated widely, mirroring in many respects the metastatic patterns seen in human cancer. This article has been written to acquaint the reader with some of the successes that have been achieved in transplanting human hematological tumours into SCID mice and to briefly describe some of the practical applications that these models of human leukemia and lymphoma have been put to. It is not intended to be an exhaustive review of the global literature but rather, to put into perspective the types of work that have been undertaken to date and to provide insights for the reader into the potential contribution that these and next generation models might make to understanding and solving the problems that surround these diseases today.

### ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

The first report describing the growth of human acute lymphoblastic leukemia (ALL) cells in SCID mice came from the laboratory of Kamel-Reid *et al.*<sup>4</sup> These workers showed that intravenous (i.v.) injection of the ALL cell line A1 into irradiated (4 Gy) SCID mice led to disseminated multiorgan disease from which animals began to die at 12 weeks. Several groups have since described the engraftment of various ALL cell lines usually without the need for preconditioning<sup>5-12</sup> and of fresh primary ALL cells<sup>5,13-16</sup> into SCID mice preconditioned with irradiation. Cesano *et al.*,<sup>5</sup> however, succeeded in establishing growth and dissemination of

T-ALL blasts from three paediatric patients in non-irradiated SCID mice. Cell lines established from two of these cases showed identical homing and progression patterns when injected into SCID mice as the patient's original cells. Isshi *et al.*<sup>8</sup> have recently shown that injection of the ALL cell line G2 together with Matrigel, an artificial extracellular matrix results in enhanced formation of subcutaneous tumours and increased levels of infiltration of bone marrow, spleen, thymus, lung and liver. As we shall see in a later section there is good evidence accumulating to suggest that the growth of patients' primary ALL cells in SCID mice is a negative prognostic indicator for any given individual patient.<sup>17,18</sup>

#### ACUTE MYELOID LEUKEMIA (AML)

Various myeloid leukemia cell lines have been successfully engrafted into SCID mice both with and without any form of preconditioning.<sup>19,22</sup> Myeloid cell lines requiring IL-3 or GM-CSF for their *in vitro* growth failed to grow in SCID mice.<sup>21</sup> Shpitz *et al.*<sup>23</sup> demonstrated that preconditioning of SCID mice with irradiation (3 Gy) or an anti-asialo GM1 antibody (to eliminate SCID mouse NK cells) resulted in improved engraftment and dissemination of the CML erythroid blast crisis cell line K562. Establishment of primary AML cells has also been attempted with variable degrees of success by several groups. Bone marrow and peripheral blood-derived myeloid leukemia cells from five of seven patients with a variety of myeloid leukemias induced patterns of leukemic infiltration that were distinct for each leukemia subtype, in cyclophosphamide-conditioned SCID mice.<sup>21</sup> Successful engraftment occurred only when cells were transplanted via the intraperitoneal (i.p.) route and cytokine support was not needed. These workers claimed that engrafted leukemic cells could be serially passaged from mouse to mouse. Chelstrom *et al.*<sup>24</sup> successfully established a proportion of primary paediatric AML cells in irradiated SCID mice without any exogenous cytokine support. Blasts from five of 12 cases produced histologically detectable disseminated leukemia, one case having a t(8;21) translocation and four cases with inv(16) karyotypic abnormalities. Moreover, only mice given leukemic blasts i.v. developed disseminated leukemia detectable histologically. Leukemia cells transplanted under the kidney capsule failed to produce overt leukemia. This is in contrast to the findings of Sawyers *et al.*<sup>22</sup> where the i.v. route was shown to be the least efficient in establishing primary AML grafts, with the kidney capsule and i.p. routes being the most efficient. Interestingly animals transplanted via the kidney capsule route did not receive preconditioning irradiation in contrast to animals receiving blasts via the i.v. or i.p. routes. Clearly discrepancies exist between different laboratories though these may be accounted for by the relatively small number of cases so far studied or by differences in the state of health or immunodeficiency of animals resident in each laboratory. Namikawa *et al.*<sup>25</sup> took a different approach and demonstrated that AML blasts could grow in fetal human bone implants in SCID mice without cytokine support or preconditioning. The surface phenotype and morphology of the leukemic cells was unaffected and blasts only grew in the human marrow graft without spread to the mouse marrow. Lapidot *et al.*<sup>26</sup> have identified the AML initiating cell, the so-called SCID leukemia-initiating cell (SL-IC), following transplantation of primary AML cells i.v. into 4 Gy-irradiated SCID mice receiving exogenous cytokine treatment with PIXY123 + hMSGF. The immunophenotype of SL-IC was shown to be CD34+CD38- and led to disease with a dissemination pattern and leukemia cell morphology similar to that seen in the original patients. CD34+CD38+ and CD34- cell fractions did not engraft. It was calculated that the peripheral blood of M1 AML patients contained one engraftment unit per 250 000 cells making SL-IC approximately 1000-fold less numerous than AML-CFU. This model therefore defines a leukemia-initiating cell which is less mature than the previously defined AML colony forming cell.

## CHRONIC MYELOID LEUKEMIA (CML)

There are relatively few reports of the growth characteristics of chronic myeloid leukemia (CML) cell growth in SCID mice. CML-derived cell lines have been successfully transplanted to both non-conditioned<sup>20,22</sup> and conditioned<sup>23</sup> SCID mice which in all cases led to disseminated disease. However, all these cell lines represent blast crises and are therefore not representative of the disease in chronic or even accelerated phase. The route of administration appeared to influence the dissemination pattern observed with the cell line KBM-5 but no changes in the levels of CD33 and CD13 expression were seen between cultured cells and those growing *in vivo*.<sup>20</sup> Sawyers *et al.*<sup>22</sup> demonstrated that bone marrow cells derived from CML patients in chronic phase showed infrequent and limited growth in SCID mice but that cells from patients in blast crisis grew much more readily in a disseminated fashion, leading these workers to suggest that the SCID mouse could form the basis for a useful assay for identifying biologically aggressive leukemias. Tsukamoto *et al.*<sup>27</sup> examined the repopulating ability of stem cells from the mobilized peripheral blood of CML patients in SCID mice carrying human bone fragments or thymus and showed that both lymphoid and myeloid lineages could be supported. However, the peripheral blood stem cells obtained in this way were not disease-free as evidenced by FISH analysis of BCR-ABL. A recent report from Wang *et al.*<sup>28</sup> has shown that both normal and leukemic hematopoietic cells could be engrafted into SCID mice from both peripheral blood and bone marrow of CML patients in chronic phase. Comparison of engraftment levels obtained in homozygous C.B-17 *scid/scid* mice and in a non-obese diabetic strain of mouse congenic for the *scid* mutation (NOD/SCID mice)<sup>29</sup> (which are not only T- and B-cell deficient but also lack functional NK cell and serum complement activity) revealed that engraftment of CML cells was more efficient in the NOD/SCID mice. The development of a SCID mouse model of CML which behaves in a biologically and clinically relevant fashion would be of great interest and of immense value in studying progression of this disease.

## MYELOYDYSPLASIA (MDS)

The author is not aware of any reported attempts in the literature to transplant human preleukemic myelodysplastic bone marrow to SCID mice, though Cotter (personal communication) has attempted this with a limited number of cases with little success. Establishment of myelodysplastic marrows which produced a pattern of disease mirroring that seen in man would be of great interest and value in following the clonal evolution of acute myeloid leukemia at both the cellular and molecular levels. Clearly more work in this area is justified, focusing particularly on the appropriate stromal microenvironment, cytokine requirements etc. necessary to ensure the successful engraftment of the relevant cell populations in the MDS marrow.

## CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Relatively few attempts have been made to establish human CLL in SCID mice. Kobayashi *et al.*<sup>30</sup> demonstrated that irradiated SCID mice injected i.p. with peripheral blood-derived CLL cells developed fatal lymphoproliferative disease. However, the tumours which grew out were biologically different from the original CLL cells and were EBV<sup>+</sup>, strongly suggesting that these tumours arose following EBV transformation of a normal lymphoid progenitor. The CLL cell lines MO1043<sup>31</sup> and D10.1<sup>32</sup> have been successfully established in non-conditioned SCID mice in both cases leading to disseminated disease.

## NON HODGKIN'S LYMPHOMA (NHL)

Human lymphomas have been notoriously difficult to grow successfully in small laboratory animals. However, recent successes have been achieved in SCID mice. Human lymphomas that have been grown in SCID mice can be broadly divided into two categories; those with and those without an Epstein-Barr virus (EBV) etiology. Both B- and T-NHL cell lines have been successfully grown and shown to disseminate widely in SCID mice<sup>33-35</sup> often with localization to sites and organs that are commonly involved in the human disease. Chang *et al.*<sup>33</sup> demonstrated the disseminated growth in non-conditioned SCID mice of a B-cell lymphoma cell line derived from a patient with what was termed large cell immunoblastic lymphoma which retained its histological appearance and killed recipient animals within 6-8 weeks. Peripheral blood mononuclear cells from this same patient with a leukemic phase to the disease also grew in SCID mice but failed to grow in culture. In contrast a T-cell lymphoma cell line grew only as small nodules in SCID mice but failed to show any metastatic spread and moreover, did not kill the animals. The observation that soluble interleukin-2 receptor (sIL-2R) is often found in the serum of patients with T-cell leukemia and lymphoma that might usefully be used to monitor disease recurrence or progression led Wasik *et al.*<sup>36</sup> to investigate the growth of a constitutive sIL-2R secreting T-cell lymphoma cell line in SCID mice. Here a clear correlative relationship was demonstrated between tumour burden or recurrence and observed serum levels of sIL-2R.

One major problem that has been encountered in attempts to grow primary human lymphoma cells in SCID hosts has been the outgrowth of EBV-transformed lymphoblastoid cell lines derived from normal host B-cells admixed with the clonal tumour population. Itoh *et al.*<sup>37</sup> transplanted 50 NHL (of both T- and B-cell lineage) to non-preconditioned SCID mice by the subcutaneous implantation of small tumour pieces. Tumours developed in SCID mice in 23 of these cases. Ten tumours were immunophenotypically and partly genotypically identical to the original primary tumours and were mainly high grade B-NHL. Most of these tumours could be serially passaged. However, widespread dissemination of these tumours to remote organs and tissues was not observed. The remaining 13 tumours that were seen, contained EBV and were likely derived from EBV-transformed normal host B-cells present in the original graft. Charley *et al.*<sup>38</sup> successfully transplanted tumour involved skin from a patient with cutaneous T-cell lymphoma to SCID mice depleted of their NK cells with anti-asialo GM1 antiserum treatment. Here the characteristic human CD4+ lymphocyte infiltrates were maintained in the grafts for the 1-month duration of the study, but dissemination outside of the grafts was not observed. Feuer *et al.*<sup>39</sup> have investigated the growth of HTLV-I associated adult T-cell leukemias, lymphoid cells from cases of HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) and asymptomatic HTLV-I seropositive patients in SCID mice. Five of 13 ATL cases, three of eight cases of HAM/TSP and one of 17 HTLV-I+ asymptomatic cases engrafted. Human lymphoblastic lymphomas developed in SCID mice injected with cells from two different cases of ATL representing outgrowth of the original ATL leukemic clone retaining monoclonal or oligoclonal integration of the HTLV-I provirus and immunophenotypes identical to the primary tumours. Later studies by Feuer *et al.*<sup>40</sup> demonstrated the importance of eliminating NK cells from the SCID host to ensure more efficient engraftment of HTLV-I and HTLV-II transformed cell lines in SCID mice. Waller *et al.*<sup>41</sup> demonstrated that primary T-cell lymphomas engrafted much more readily into SCID mice that had been previously engrafted with human fetal tissues. Two clonal T-cell lymphomas with immunophenotypes and genotypes identical to the original tumours grew after injection of primary lymphoma cells directly into human fetal thymus xenografts. Successful serial passage of these lymphomas required the

presence of a human lymphoid microenvironment and was enhanced by depleting mice of their NK cells either by irradiation or treatment with anti-asialo GM1 antiserum Waller *et al.*<sup>42</sup> have also investigated the growth in SCID mice of four post-transplant lymphomas that lack the expression of the usual markers associated with EBV infection. Two of these were T-cell lymphomas that were EBV+. Again growth of lymphomas was facilitated in animals previously xenografted with human fetal thymus tissue and depleted of NK cells.

There has been much interest focused on the use of SCID mouse models to delineate the etiology of the EBV-associated immunoblastic lymphomas often seen post-transplant or in other immunosuppressed states. Several groups have clearly shown that peripheral blood mononuclear cells (PBMC) from EBV seropositive donors transplanted intraperitoneally into SCID mice frequently give rise to immunoblastic lymphomas reminiscent of those seen in immunosuppressed patients post-transplant.<sup>43-48</sup> In contrast, PBMC from EBV seronegative donors do not give rise to tumours in SCID mice. Boyle *et al.*<sup>49</sup> addressed the issue of active and latent infection with EBV and the outcome this had on lymphomagenesis in SCID mice following transfer of human PBMC and found that the latent period to lymphoma appearance was much reduced (4-7 weeks) in active infection where EBV was administered together with seropositive or negative human PBMC compared with latent infection (11-14 weeks). The tumours arising following transfer of PBMC from EBV positive donors generally secrete oligoclonal or monoclonal immunoglobulin, contain detectable EBV DNA and usually do not show the typical chromosomal abnormalities associated with EBV-induced lymphomas in man, though at least one exception with a t(5:14) translocation has been reported.<sup>50</sup> There has been shown to be a marked variation in the ability of PBMC from different EBV+ individuals to induce lymphomas in SCID mice and this has been at least partly attributed to the state of activation of the EBV genome in the transplanted host B-cells. It has been generally suggested that the variable patterns of lymphomagenesis observed for different individuals may be explained by the lower levels of specific immunity to EBV in those individuals that have a high incidence of tumour formation. PBMC from patients with Sjogrens syndrome (SS) at increased risk for lymphoma development in the salivary glands, also produced immunoblastic lymphomas when injected into SCID mice.<sup>51</sup> However, these tumours did not arise in the salivary glands and lacked many of the characteristics associated with SS lymphomas and contained high levels of EBV DNA and EBV-associated antigens. Two groups have reported that injection of purified B-cells from EBV seropositive donors into SCID mice does not result in the formation of tumours.<sup>43,52</sup> Similarly, treatment of SCID mice with the T-cell suppressive agent cyclosporine following transfer of PBMC from EBV+ donors reduces the incidence of tumour formation in latent infection but not active infection.<sup>44,48</sup> These findings indicate the importance of T-cells or a T-cell factor(s) in the lymphomagenesis process following administration of latently infected PBMC. It has been shown that CD4+ and CD8+ T-cell subpopulations are both capable of providing the putative factor(s) necessary for EBV+ B-cell expansion and tumour progression.<sup>53</sup> Recent studies by Rochford and Mosier<sup>54</sup> have shown that B-cell lymphomas arising in SCID mice injected with PBMC from EBV+ donors could be divided into two subpopulations on the basis of their levels of CD23 and CD38 expression. The subset of lymphoma cells with intermediate expression levels of both antigens (CD23<sup>int</sup> CD38<sup>int</sup>) had a high proliferative index and a low level of immunoglobulin secretion whilst cells with low expression of CD23 and high expression of CD38 (CD23<sup>lo</sup> CD38<sup>hi</sup>) had a low proliferative index but a high level of immunoglobulin secretion. Latent EBV transcripts were found only in cells with the CD23<sup>int</sup> CD38<sup>int</sup> phenotype whereas lytic transcripts and transforming virus were present only in the CD23<sup>lo</sup> CD38<sup>hi</sup> subset. It was further shown that short-term cell lines generated from the CD23<sup>int</sup> CD38<sup>int</sup> subset when transplanted into SCID mice gave rise to secondary tumours predominantly with a CD23<sup>lo</sup>

CD38<sup>hi</sup> phenotype which suggested to the authors that the CD23<sup>int</sup> CD38<sup>int</sup> lymphoblastoid cells are precursors of the further differentiated plasmacytoid CD23<sup>lo</sup> CD38<sup>hi</sup> lymphoma cells.

### MULTIPLE MYELOMA (MM)

Both fresh bone marrow/peripheral blood-derived multiple myeloma (MM) cells<sup>55,56</sup> and cell lines<sup>11,57,58</sup> have been successfully transplanted to SCID mice. Some MM cell lines such as IM9 and HS-SULTAN when injected i.v. into non-conditioned SCID mice lead invariably to fatal disseminated disease with tumour cells found in blood, spleen, liver, bone marrow, brain, spine and kidney.<sup>11</sup> Other MM cell lines such as ARH-77 only grew if the mice were irradiated prior to i.v. injection of cells.<sup>57</sup> Mice injected with ARH-77 cells developed osteolytic lesions in the vertebrae and bones of the skull strongly resembling advanced MM in man. These animals also developed hind leg paralysis as a result of infiltrating MM cells compressing the spinal cord. Fresh MM cells derived from bone marrow/peripheral blood of two of six patients also grew in unconditioned SCID mice with plasma cells detected in mesenteric fat tissue around pancreas and spleen<sup>55</sup> with one of these animals having demonstrable monoclonal human immunoglobulin in the serum. Feo-Zuppari *et al.*<sup>56</sup> showed that i.p. injection of fresh highly purified MM cells into SCID mice gave a high engraftment rate. Monoclonal circulating human immunoglobulin was found only in animals receiving purified MM cells, animals receiving unfractionated myeloma marrow showed polyclonal immunoglobulin secretion.

### HODGKIN'S DISEASE

Both Hodgkin's disease (HD)-derived cell lines<sup>59-61</sup> and primary HD-involved tissue<sup>6</sup> have been successfully transplanted to SCID mice. Intravenous injection of the HD cell lines L540 and L540cy into non-conditioned SCID mice led to the development of disseminated tumour growth with the histological appearance of CD30+ anaplastic large cell lymphomas. Lymph nodes were the preferred sites of tumour growth. Other HD cell lines (L428 and KM-H2) when injected i.v. failed to grow. Kapp *et al.*<sup>62</sup> transplanted HD-involved tissue from 13 HD patients beneath the renal capsule of SCID mice and observed tumours arising in three cases. The data suggested that these tumours might have been derived from EBV superinfected Hodgkin and Reed-Sternberg cells or from EBV infected bystander cells.

### BIOLOGICAL BEHAVIOUR

#### Homing patterns and phenotypic stability

Many workers comment that the pattern of disease distribution seen in SCID mice injected with human leukemia or lymphoma cells parallels the natural distribution pattern often seen in human leukemia/lymphoma patients.<sup>12</sup> In the majority of cases studied the bone marrow appears to be the first organ to be infiltrated.<sup>10,18,63</sup> Infiltration of the central nervous system is also commonly encountered particularly of the meninges and subarachnoid space<sup>10</sup> and the dorsal root ganglia leading frequently to paralysis.<sup>64,65</sup> Leukemia and lymphoma cell lines injected intravenously can form solid tumours in almost any organ with individual lines showing different predilections for different organs. Different routes of administration can also lead to different dissemination patterns.<sup>63</sup> Bashir *et al.*<sup>66</sup> has provided some limited evidence that LFA-1 expression by an EBV transformed lymphoma cell line plays a role in the homing of these cells to the brain via interactions with ICAM-1 expressed by endothelial cells within the vasculature of the brain. The current evidence thus points to a likely involvement of specific homing

mechanisms, the unravelling of which may lead to important insights into understanding the behaviour and characteristics of these tumours in man.

The immunophenotypic stability of leukemia and lymphoma cells engrafted into SCID mice has also been reported on by several groups. Kamel-Reid *et al.*<sup>9</sup> described the induction of CD10 expression on the CD10 – pre-B cell leukemia cell line G2 growing in the thymus of irradiated SCID mice. Further studies by Ishii *et al.*<sup>67</sup> showed that induction of CD10 expression in G2 cells occurred in hematopoietic tissues at the time of infiltration and proliferation into these compartments and was followed later by expression on leukemia cells found in all peripheral organs which suggested to these authors an association with disease progression. Uittenbogaart *et al.*<sup>12</sup> have described striking immunophenotypic changes in T-cell leukemia cell lines growing in SCID mice. In particular the T-ALL cell line CEM showed upregulation of CD3 and CD8 expression suggesting that some maturation had occurred. Two other cell lines SUP-T3 and MOLT-4 showed changes in CD45RA expression which were also consistent with maturation. Downregulation of expression of a variety of cell surface markers in leukemia cells following transfer to SCID mice has also been described which includes CD7<sup>68</sup> (Flavell, unpublished results) and CD38, CD22 and CD19 (Flavell, unpublished results). Moreover, we have shown that the level of CD19, CD38 and CD22 expression by the Burkitt's lymphoma cell line Ramos is determined by the site at which the tumour is growing in the animal, with tumours growing in the kidney showing the lowest levels of expression. These observations taken collectively clearly demonstrate that the SCID mouse microenvironment can influence the expression of differentiation molecules on the leukemia/lymphoma cell surface and thereby possibly modify their biological behaviour.

#### Prognostic factors

Kamel-Reid *et al.*<sup>18</sup> first demonstrated a relationship between the rate of growth in irradiated SCID mice of fresh ALL cells taken from paediatric patients with pre-B cell ALL and the time to relapse following therapy. There was a good correlation between the rapidity with which the ALL grew in the SCID mouse and the time to relapse for any individual patient. Uckun *et al.*<sup>69</sup> went a step further and studied the growth of bone marrow-derived pre-B ALL cells from 42 children with newly diagnosed high risk leukemia and found that the occurrence of overt leukemia in the mice was a highly significant predictor of relapse with a 21.5-fold greater chance of relapse in patients whose ALL cells grew compared with those that did not.

#### Multi-drug resistance

Recent reports describe the growth of drug-resistant leukemia and lymphoma cell lines in SCID mice. Beran *et al.*<sup>19</sup> showed that the growth of drug sensitive parental human myeloid leukemia cell lines HL60, K562 and KBM3 in SCID mice was identical to that of the same cell lines rendered resistant *in vitro* to amsacrine, vincristine, hycamptamine, methotrexate or doxorubicin. We ourselves have shown that the daunorubicin-resistant human T-ALL cell line HSB-2dauno 17.5 grows in SCID mice in an identical fashion to the drug-sensitive parent cell line HSB-2 (Flavell, unpublished results). A drug-resistant multiple myeloma cell line MDR8226/C1M expressing the p-glycoprotein has also been successfully grown in SCID mice and shown to be more resistant *in vivo* to doxorubicin than the parental 8226 cell line.<sup>70</sup> Lacerda *et al.*<sup>71</sup> have shown that SCID mice bearing the methotrexate (MTX) transport resistant T-ALL cell line CCRF CEM are still sensitive to trimetrexate (TMTX) used alone or together with leucovorin rescue. Interestingly, methotrexate resistant CCRF CEM cells treated *in vivo* with TMTX appeared to regain sensitivity to MTX and show an increased transport of MTX into the

cell. There is also some evidence that vincristine- and adriamycin-resistant Burkitt lymphoma cells growing in SCID mice still retain their sensitivity for an anti-CD19 blocked ricin immunotoxin (B4-br) providing an encouraging indication that multi-drug resistant tumour cells will still remain sensitive to immunotoxin-mediated killing.<sup>72</sup> Clearly there is likely to be much gained from developing new models of multi-drug resistance of leukemia and lymphoma in SCID mice which will bring us to a closer understanding of this clinically important phenomenon. An important goal that should be pursued in the coming years is the development of an *in vivo* model of MDR which parallels as closely as possible the clinical picture as seen in man.

### THERAPY MODELS

To date the single most commonly exploited application for SCID mouse models of human leukemia and lymphoma has been for investigation of experimental therapeutic modalities. The next section summarizes examples of such studies.

#### Immunotherapy

Murine monoclonal antibodies which recognize specific cell surface molecular structures have been shown to exert anti-tumour effects in human leukemia/lymphoma bearing SCID mice. There are at least two possible broad mechanisms operating here either or both which may contribute, these being (1) recruitment of SCID mouse host effector mechanisms such as NK cells or complement (both of which are intact in SCID mice) and/or (2) direct cellular signalling resultant to the binding of antibody to ligand. Anti-CD7,<sup>3</sup> anti-CD19,<sup>65,74</sup> anti-CD38,<sup>35</sup> anti-CD22,<sup>75</sup> anti-CD40,<sup>76</sup> anti-CD95 (APO-1),<sup>77</sup> anti-CDw126 (IL-4R)<sup>78</sup> and anti-CD54 (ICAM-1)<sup>79</sup> murine antibodies have all been shown to exert specific anti-tumour effects. However, there are other instances where the same or similar antibodies have had little or no anti-tumour effect.<sup>80,81</sup> Taking a different approach Pohl *et al.*<sup>82</sup> produced an anti-anti-idiotypic antibody which recognized the CD30 molecule and exerted anti-tumour effects in SCID mice bearing the CD30<sup>+</sup> Hodgkin's cell line L540.

Adoptive transfer studies have also demonstrated the anti-tumour effects of a variety of human effector cells. Cesano *et al.*<sup>83</sup> showed that the adoptive transfer to SCID mice of the human killer T-cell clone T-ALL-104 given together with IL-2 at the same time as challenge with the monocytic leukemia cell line U937 significantly prolonged the survival of mice. Total eradication of tumour was however only achieved when repeated injections of T-ALL-104 cells were given. Malkovska *et al.*<sup>84</sup> showed that cytotoxic v<sub>9/vδ2</sub> cells generated by *in vitro* incubation of normal peripheral blood lymphocytes with the human B-cell lymphoma cell line Daudi, when adoptively transferred to SCID mice afforded some protection against the growth of Daudi lymphomas in these animals. This effect was shown to be Daudi specific and was not mediated by murine NK cells.<sup>85</sup> Lu and Negrin<sup>85</sup> used cytokine induced killer (CIK) and lymphokine activated killer (LAK) cells for the systemic therapy of SCID mice bearing the human B-cell lymphoma cell line SU-DHL4 and showed that CIK cells were significantly better at conferring protection against SU-DHL4 lymphoma growth in SCID mice.

#### Oligonucleotide therapy

Some positive results have been obtained suggesting a positive therapeutic benefit of antisense therapy in SCID mice bearing human leukemias or lymphomas. Ratajczak *et al.*<sup>87</sup> demonstrated that a C-MYB antisense oligonucleotide significantly prolonged the survival of SCID mice



injected with the myeloid leukemia cell line K562 compared with untreated control, sense or scrambled sequence C-MYB oligonucleotides. Using a 26-mer BCR-ABL antisense oligonucleotide Skorski *et al.*<sup>88</sup> were able to demonstrate a significant prolongation in survival of SCID mice bearing the transplanted CML blast crisis cell line BV173 with the Philadelphia translocation compared with untreated or sense treated oligonucleotides. Cotter *et al.*<sup>89</sup> showed that SCID mice injected with t(14;18) BCL2 deregulated DoHH2 human B-cell lymphoma cells that had been previously treated *in vitro* with an antisense oligonucleotide to the first open reading frame of the BCL2 gene showed downregulation of BCL2 protein and did not develop tumours. This contrasted sharply with untreated DoHH2 cells or cells treated with sense and nonsense oligonucleotides all of which gave rise to lymphomas in the SCID mice.

#### Gene therapy

Cook *et al.*<sup>90</sup> have described the first gene therapy study to be undertaken in SCID mice by delivering the gene for diphtheria toxin (DTA) coupled via polylysine to an adenovirus carrier to EBV transformed human LCL cells engrafted in the SCID mice. It was shown that DTA gene treatment prolonged the survival of animals beyond that seen in controls.

#### Immunotoxins

SCID mouse cell line models of leukemia and lymphoma have been used by several groups to investigate the therapeutic efficacy of immunotoxins. Immunotoxins with a variety of anti-leucocyte specificities, containing ricin A chain,<sup>34</sup> blocked ricin,<sup>91</sup> pokeweed antiviral protein<sup>64,80</sup> or saporin,<sup>73</sup> have been investigated in T-<sup>10,92,93</sup> and B-cell<sup>6,65,75,80,91,93</sup> malignancies, Hodgkin's lymphoma<sup>94</sup> and anaplastic large cell lymphoma.<sup>95</sup> All studies reported significant therapeutic benefits from immunotoxin treatment with prolongation in survival and apparent cures in a proportion of treated animals. However, in some studies naked antibody also exerted an anti-tumour effect, though this was generally less pronounced than that obtained with immunotoxin.<sup>6,65,73,93</sup> In other studies naked antibody exerted no therapeutic effect.<sup>80,95</sup> Reports have recently appeared demonstrating that treatment with combinations of two immunotoxins in SCID mouse models of B-cell lymphoma is therapeutically superior to treatment with each individual immunotoxin<sup>34</sup> or immunotoxin plus antibody.<sup>35</sup>

#### Combination immunotoxin/chemotherapy studies

The consensus view that immunotoxins are likely to be used as an adjuvant therapy in the setting of minimal residual disease has led some workers to investigate the therapeutic efficacy in SCID mice of small molecule cytotoxic drugs used in combination with an immunotoxin. Uckun *et al.*<sup>64,80</sup> have shown that when an anti-CD19 immunotoxin (B43-PAP) and cyclophosphamide are used in combination, the therapeutic outcome is superior to that obtained when either the immunotoxin or drug are used alone in a SCID mouse model of pre-B-cell ALL. A similar observation was made with the same combination used in the treatment of a different leukemia cell line also in SCID mice.<sup>96</sup> However, in neither of the above studies were complete cures obtained in all animals. Most impressive of all, were the results obtained by Ghetie *et al.*<sup>97</sup> who using a combination of two ricin A chain immunotoxins (anti-CD19 and anti-CD22) together with one of three different cytotoxic drugs (doxorubicin, cytoxan or camptothecin) obtained cures in all SCID mice bearing the human B-cell lymphoma cell line Daudi. Treatment with the immunotoxin cocktail alone or with any of the three cytotoxic drugs did not result in cures.

#### Miscellaneous therapies

Honma *et al.*<sup>98</sup> investigated the anti-leukemic effect of herbimycin A in SCID mice bearing Philadelphia+ and Ph- leukemias. Herbimycin A reduces intracellular phosphorylation by

BCR-ABL tyrosine kinase and therefore is predicted to preferentially inhibit the growth of Ph+ leukemias. This was borne out in this study where herbimycin A prolonged the survival of mice with Ph+ but not Ph- leukemias. Recently, Uckun *et al.*<sup>17,69</sup> reported on the activity of the synthetic topoisomerase I inhibitor topotecan in SCID mice bearing pre-B-cell leukemias and demonstrated a potent anti-leukemia effect. In another recent study Schwarz *et al.*<sup>99</sup> have demonstrated that IL-4 inhibits growth of the human B-cell lymphoma cell line Daudi in SCID mice.

### FUTURE PROSPECTS

The accumulating experience with the various SCID mouse models of human hematological malignancies indicates that they provide useful and arguably relevant systems in which a range of biologically and clinically relevant problems can be addressed as evidenced in this short review. Whilst the potentials are great, there are, it must be realized, limitations to these models which must be taken into consideration when interpreting results. The vast majority of studies have to date been undertaken with cell lines which may not represent the true behaviour of their parent tumours. There is a need to develop models utilizing freshly obtained primary patient material, and as we have seen several groups are already making successful attempts in this area. SCID mice also possess functionally normal or near normal natural killer (NK) cells which present a barrier to efficient engraftment, particularly with primary patient material. Some workers have successfully overcome this barrier by depleting SCID mice of their NK cells using irradiation, cytotoxic drugs (cyclophosphamide) or anti-NK cell antibodies (anti-asialo GM1 antiserum). However, these procedures can be expensive, logistically difficult to undertake in the context of material availability and moreover, serve to introduce another variable into experimental results. The recent development of non-obese diabetic (NOD)/LtSz-SCID mice<sup>100</sup> which are not only T- and B-cell deficient but also lack functional NK cells and also have a deficiency in serum complement components may prove more appropriate for the development of future models of human hematological malignancies and some workers have already begun exploring this possibility.<sup>28</sup> The lack of a functional immune system may also have an important bearing on disease pathogenesis following transplantation of human leukemia/lymphoma cells. This is likely to be particularly so in Hodgkin's disease where putative Hodgkin's tumour cells can be successfully transplanted but without the development of a host stromal cell response so characteristic of the disease in man. When investigating therapies involving antibody targeting against human leukocyte differentiation molecules on the tumour cell surface (e.g. CD19, CD7 etc.) it must be remembered that unlike human patients, SCID mice do not possess cells expressing these antigens which in man would present a barrier to therapy due to removal of the therapeutic by normal leukocytes and thus significantly influencing pharmacodynamics and kinetics. Despite these shortcomings, SCID mice have provided new and potentially useful models which are likely to continue making positive contributions to a variety of diverse areas in the field of hemato-oncology.

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